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THE VIABILITY AND GROWTH OF B. TYPHOSUS IN BILE

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During the past twenty-five years there has been much discussion regarding the effects of bile and bile salts on members of the colon-typhoid group of organisms. Some workers suggest that the fluid is germicidal toward these forms, others think that it is not inhibitive and a third group believes that it is unfavorable at first and that later the organisms most fit and which have survived its influence propagate slowly.

Without discussion of the exact limitations of alteration of bile to render it most favorable to the growth of the organism or of the source from which it was taken for experimental purposes, it may be stated that the following authors believe that at least bile does not inhibit the proliferation of B. typhosus nor of B. coli: Von Fütterer,[1] Leubuscher,[2] Corrado,[3] Fraenkel and Krause,[4] Babes,[5] Meyerstein,[6] Hirokawa[7] and Nichols.[8] On the other hand, Mosse,[9] Talma,[10] Pies,[11] Fornet,[12] and Knick and Pringsheim[13] believe that it is bacteriostatic. Dunschmann[14] finds that sodium taurocholate favors the proliferation of B. typhosus while sodium glycocholate depresses it. The third point of view, namely, that bile inhibits slightly while the surviving forms grow slowly, has been held by Jordan,[15] who states that bile hinders the growth of approximately one third of the viable cells of B. coli, and Ecker,[16] who believes that many living cells of B. typhosus refuse to multiply in bile and that bile salts are distinctly unfavorable to it.

An interesting by-product relative to the effect of bile on intestinal bacteria is to be noted in the use of bile mediums for examination of water and milk for the detection of members of this group in sanitary analysis and also for

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[1] Münch. med. Wchnschr.. 1888, 35, p. 315.
[2] Ztschr. f. klin. Med., 1890, 17, p. 472.
[3] Atti della Accademia Medica de Roma, 1891.
[4] Ztschr. f. Hyg. u. Infektionskr., 1889, 32, p. 97.
[5] Berlin. klin. Wchnschr., 1899, 17, p. 362.
[6] Centralbl. f. Bakteriol., I, O., 1907, 44, p. 434.
[7] Centralbl. f. Bakteriol., I. O., 1909, 53, p. 12.
[8] Jour. Exper. Med., 1916, 24, p. 495.
[9] Ztschr. f. klin. Med., 1899, 36, p. 527.
[10] Centralbl. f. Bakteriol., Ref., 1901, 29, p. 367.
[11] Arch. f. Hyg., 1907, 62, p. 107.
[12] Arch. f. Hyg., 1907, 60, p. 134.
[13] Deut. Arch. klin. Med., 1911, 101, p. 137.
[14] Ann. de l'Inst. Past., 1909, 23, p. 29.
[15] Jour. Infect. Dis., 1913, 12, p. 326; Trans. 15th. Internat. Cong. Hyg., ii, p. 48.
[16] Jour. Infect. Dis., 1918, 22, p. 95.

blood cultures in typhoid fever. Limbourg,[17] Fraenkel and Krause,[4] Grünbaum and Hume,[18] Matzuschita,[19] Jackson,[20] MacConkey,[21] MacKonkey and Hill [22] and Dunschmann [14] have advocated the use of bile and bile salts for examination of B. coli and its near relatives. Such mediums are now being abandoned, however, following the reports unfavorable in character by Jordan,[16] Jordan, Russell and Zeit,[23] Longley and Baton,[24] Cumming,[25] Obst,[26] Tonney, Caldwell and Griffin.[27]

Technic.—Experiments were attempted to learn (a) whether the strain of B. typhosus used in this work is viable in bile, and (b) whether it can multiply in such medium freely. The strain of B. typhosus used is that denominated as "No. 3" in this laboratory and was isolated originally from a carrier.

As opportunity allowed, there were obtained 7 samples of bile of which 5 were of human origin and one each from rabbit and beef. Those from human necropsy had the following histories: The first was from a case of carcinoma of the stomach; the second was from an old man with chronic passive congestion of the liver and no other distinctive lesions; the third was from a case of carcinoma of the pancreas with infection of the gallbladder. No information was obtained regarding the other two.

Of each sample 15 c c were placed in a tube and autoclaved for 15 minutes at 15 pounds' pressure since the material was not sterile when received. The rabbit bile was an exception in amount on account of its small volume, which was but 2 c c, and was put in an agglutination tube. When again cool, each was inoculated with two 2 mm. loopfuls of a 24-hour broth culture of B. typhosus. The cotton plugs were cut off even with the top of the tube and thrust down the bore sufficiently far so that a sterile rubber stopper could be placed in the mouth of the container to prevent evaporation during subsequent incubation. These preparations were then incubated at 37 C. with streaks made from them intermittently to determine whether inoculated organisms were still living. No attempt was made at first, therefore, to observe possible increase in numbers.

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[17] Ztschr. f. phy. Chem., 1889, 13, p. 196.

[18] Brit. Med. Jour., 1902, 1, p. 1473.

[19] Cent. f. Bakteriol., Ref., 1902, 31, p. 505.

[20] Biol. Studies by Pupils of W. T. Sedgwick, 1906 p. 292.

[21] Thompson-Yates Laboratory Reports, 1900, 3, p. 41; Jour. Hyg., 1908, 8, p. 322.

[22] Thompson-Yates Laboratory Reports, 1901, 4, p. 151.

[23] Jour. Infect. Dis., 1904, 1, p. 641.

[24] Jour. Infect. Dis., 1907, 4, p. 397.

[25] Hyg. Lab. Bull., U. S. Pub. Health Serv., 1915, 104.

[26] Jour. Bacteriol., 1916, 1, p. 73.

[27] Jour. Infect. Dis., 1916, 18, p. 239.

At the termination of this experiment, streaks made from all of the cultures in bile were heavily positive for B. typhosus, and they were pure. Lack of time did not allow the experiment to continue a sufficient length of time for us to learn the period of possible viability of typhoid in the bile used. The results for each sample are given below.

TABLE 1

RESULTS OF EXPERIMENT

Number	Origin	Observation Period	Outcome
1	Human	160 days	Positive
2	Human	160 days	Positive
3	Rabbit	160 days	Positive
4	Beef	141 days	Positive
5	Human	126 days	Positive
6	Human	126 days	Positive
7	Human	126 days	Positive

Therefore, this strain of B. typhosus proved to be extremely long lived in human, ox and rabbit bile.

Next, knowledge concerning the rate of growth of B. typhosus in beef bile was sought with the following procedure: A stock of bile made up of the contents of four gallbladders was obtained from a local slaughter house and while still warm was placed in flasks in 100 c c quantities and autoclaved at 15 pounds' pressure for 15 minutes. It is interesting here to note the fact that this process of sterilization changed the hydrogen-ion concentration from P_H 7.2 in the raw bile to P_H 8.4 after cooking. Determinations were made according to the colorimetric method of Clark and Lubs.[28] A flask of bile was inoculated with 0.5 c c of a 24-hour broth culture of B. typhosus and plates poured as soon as possible after thorough shaking in order to estimate the initial number of organisms per c c. Dilutions were carried out according to the methods generally accepted for counting milk and water and 3 plates made of each. This flask was placed in the incubator at body temperature and left there during the course of the experiment. Thereafter, plate determinations with plain beef extract agar for the count per c c of the bile were carried out in ½, 1, 1½, 2, 3, 4, 6, 8, 24 and 48 hours. Most thorough agitation was given the test flask and dilution blanks before final plate inoculums were removed. Petri dishes thus poured were incubated at 37 C. for 24 hours in an inverted position and then counted.

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[28] Jour. Bacteriol., 1917, 2, p. 1, p. 109 and p. 191.

TABLE 2

COUNTS OF SAMPLE OF BEEF BILE

Period	Count per C c
Immediate	155,000
½ hour	100,000
1 hour	70,000
1½ hours	67,500
2 hours	60,000
3 hours	47,500
4 hours	40,500
6 hours	43,000
8 hours	59,000
24 hours	292,000
48 hours	2,600,000

To serve as a partial check to this, beef broth was prepared, placed in 100 c c portions in Erlenmeyer flasks, sterilized in the same intensity and with reaction altered also to P_H 8.4. However, the broth was not used for 3 days, during which time the P_H had changed to 7.9, as may be expected to happen with any batch of medium. It was used without further alteration. Like technic was followed except that count determinations concluded with one at 12 hours when turbidity was becoming very evident.

TABLE 3

NUMBERS PER C C OF BEEF BOUILLON

Period	Count per C c
Immediate	200,000
½ hour	180,000
1 hour	217,500
1½ hours	210,000
2 hours	265.000
3 hours	575,000
4 hours	1,660,000
6 hours	26,400,000
8 hours	64,000,000
12 hours	187,500,000

It was realized that to be of greatest value, a check in beef broth should have a like reaction. Moreover, it was felt that possibly the lags were greater than they would be if both flasks were kept at or near 37 C. throughout the experiment. Therefore the series of count determinations was repeated with the broth on this second occasion in like P_H value to that of the bile which again was 8.4. The bile was taken from another lot of material collected at a different time and in both series was 48 hours old when manipulated. Also like the first, the second had been sterilized while still warm from the slaughter house. The flask of beef broth and that of bile had both been in the incubator over night and, except for the few moments while the initial dilution was being prepared at each period, was kept there throughout the experiments. Unfortunately, a small number of the water flasks used in making dilutions proved to be contaminated because of imperfect sterilization—therefore the blanks which will be noted in table 4. The results in parallel are as herewith; figures are the quantity of organisms per c c.

TABLE 4

RESULTS OF SERIES OF COUNT DETERMINATIONS MADE WITH BROTH

Time	Bile	Broth
Immediate	176,000	....
½ hour	140,000	190,000
1 hour	106,000	192,000
1½ hours	133,000	297,000
2 hours	....	427,000
3 hours	....	1,160,000
4 hours	175,000	7,500,000
6 hours	530,000	48,500,000
8 hours	2,360,000	115,000,000
12 hours	3,975,000	430,000,000

It will be noted from these figures that B. typhosus in bile first passes through a period of germicidal action. The mortality in the first experiment during this phase amounted to nearly 74%, while in the second it approximated 40%. The second period is characterized by an exceedingly long lag and this in turn is followed by slow but progressive growth which, however, never increases to the point of production of turbidity. The outcome with B. typhosus is much like that of Jordan's [15] with B. coli and the conclusions are in part identical with those of Ecker.[16]

The strain of B. typhosus which was originally isolated from a carrier is exceedingly long lived in human, beef and rabbit bile. Introduced into sterile beef bile, it is subjected to marked bactericidal activity on the part of the medium which brings about the death of a large fraction of the living forms. This is followed by an evident lag and in turn is succeeded by relatively slow but progressive proliferation.